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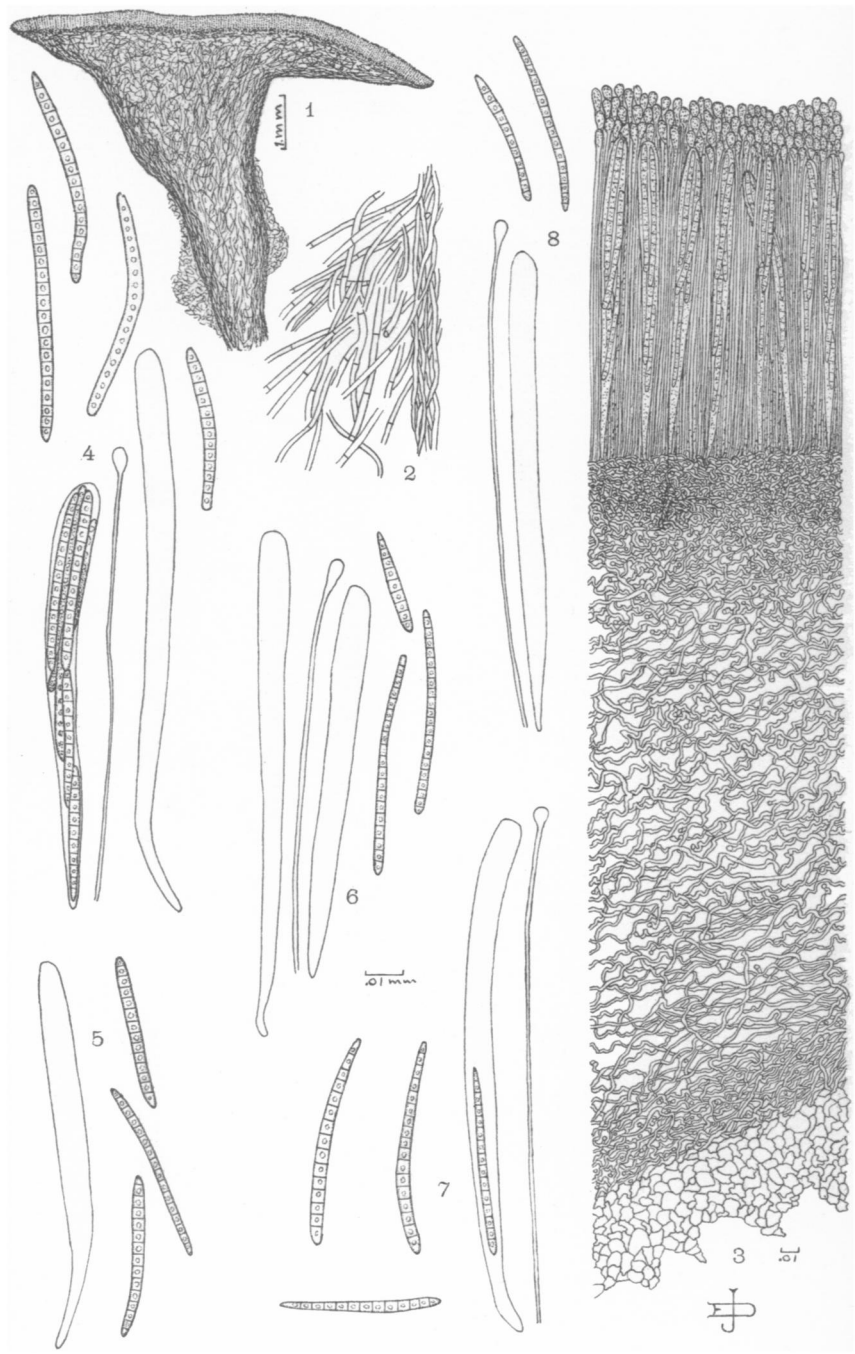
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HOLWAYA GIGANTEA (Peck) Durand

Studies in North American Discomycetes. I. The Genus *Holwaya* Sacc.

BY ELIAS J. DURAND

(WITH PLATE 26)

In the autumn of 1895, I collected a discomycete on a large prostrate log in the vicinity of Ithaca, which I determined at the time as *Holwaya ophiobolus* (Ellis) Sacc., since it agreed in all respects with the specimens in Ellis's N. A. F., No. 996. My plants were accompanied by a Hyphomycetous fungus referable to genus *Graphium*. The possible connection of the two forms suggested itself to me at the time, but I thought no more about it until the winter of 1898, when Professor Burt read a paper before the Society for Plant Morphology and Physiology, at Ithaca, on the subject, "Is there a Basidiomycetous stage in the life history of some Ascomycetes?" Since that time I have been collecting notes and observations on which the present paper is based.

The genus *Holwaya* was described by Saccardo in 1889, to include a Bulgariaceous discomycete externally resembling *Bulgaria inquinans* (Pers.) Fr., but differing from it in the possession of filiform, multiseptate spores. The genus was based on *Bulgaria ophiobolus* Ell., which had already been described from material collected in Iowa by Holway, and distributed in Ellis's N. A. F., No. 996. According to the original description the plant is "composed of two layers, separated by a gelatinous stratum." This gelatinous character was not evident in my material (Fig. 8).

A second species in the genus, *H. tiliacea* E. & E., was described in 1897, from material collected in Canada, on the bark of dead *Tilia*. The differences between this and the first species are not very evident from the descriptions. In 1899, Professor Burt sent me specimens collected on basswood logs, at Middlebury, Vt., and determined by Mr. Ellis as *H. tiliacea* E. & E. In an accompanying note Professor Burt said that he did not see

why *H. ophiobolus* was not the same thing. After a careful comparison of this material with that in the N. A. F., No. 996, I must confess that I can detect no difference. They are alike in all essential respects (Fig. 7).

In 1878, Dr. Peck described a discomycete collected on rotten maple bark, which he called *Patellaria leptosperma* Pk. Saccardo later transferred this species to *Lecanidion* on account of its hyaline spores. It is interesting to note that some of Professor Burt's material already mentioned was sent to Dr. Peck, who named it *Lecanidion leptospermum* (Pk.) Sacc. Furthermore, I recently sent some of my 1895 collection to Dr. Peck, with the request that he compare it with his species. He referred it to *Patellaria leptosperma*. Dr. Peck was also good enough to send me specimens of this species, collected at Copake, N. Y. They agree in every respect with the specimens mentioned in the preceding paragraphs (Fig. 6).

But this is not all. In 1893, there was published under the name *Chlorosplenium Canadense* E. & E. the description of a discomycete collected in Canada, on rotten basswood bark, by Prof. John Dearness. According to the descriptions this seems to differ from *Holwaya* principally in the tomentose stem. Mr. Dearness very kindly sent me recently three specimens from as many gatherings, all of which evidently belong to the same species. One of these specimens was a part of the original material from which *C. Canadense* E. & E. was described. Although the specimen is too young to show spore characters, in all other respects it agrees with specimens of *Holwaya ophiobolus*, *H. tiliacea*, and *Lecanidion leptospermum*. The original description of the spores shows that they, also, are alike. The other specimens from Mr. Dearness exhibit these characters plainly (Fig. 5). With regard to the tomentose character of the stem, a study of specimens has revealed the presence of the olive-brown tomentum on the stems of all examined. In the structure of the sterile layers of the ascoma we find further evidence of the specific identity of these plants. I have made careful paraffin sections of specimens from Professor Burt, from Ellis's N. A. F., No. 996, and of plants collected at Ithaca in 1900, and find that the structure is exactly the same in all. (Fig. 3). In the light of what has been said, therefore, I cannot

but regard *Holwaya ophiobolus* (Ell.) Sacc., *H. tiliacea* E. & E., *Lecanidion leptospermum* (Pk.) Sacc., and *Chlorosplenium Canadense* E. & E. as one and the same species.

Turning now to the subject of a conidial phase of this species, I find that a plant called *Stilbum giganteum* Peck was described in 1871. Saccardo, however, transferred it to the genus *Graphium*. The first mention of this plant as a possible imperfect stage of an ascomycete was made by Dr. Peck in his thirty-first report written in 1878, where he said: "I find this associated with *Patellaria leptosperma* Pk. in such a way as to suggest the probability, at least, that it is a form of the latter species." Ellis and Everhart in their descriptions of *Chlorosplenium Canadense* and *Holwaya tiliacea* in each case call attention to the fact that *Coryne Ellisii* Berk. (*Stilbum magnum* Pk.) was found associated with the species, of which it might be a conidial stage. As a matter of fact the association of the two forms has been noted in the great majority of the records of occurrence. The fact that it has not been mentioned in the other cases does not, by any means, go to show that they were not growing together, for such association might have been overlooked. Regarding "*Stilbum magnum* Pk.," Dr. Peck assures me that he never has published such a species, but that the occurrence of the name in the literature is probably due to a slip of the mind or pen, *Stilbum giganteum* being intended in each case.

Coryne Ellisii Berk. was published in 1873 from material found by Ellis, on basswood logs, at Potsdam, N. Y. No asci were present in the original specimens, but the plant was referred to *Coryne* probably on account of a superficial resemblance to the members of that genus. Indeed, Berkeley remarked that an examination of fresh material was desirable. The matter was further greatly complicated when Massee, in 1894, redescribed the type of *Coryne Ellisii*, making it one of the types of a new genus of the Basidiomycetes, called *Dacryopsis*. Our plant was given the name *D. Ellisiana* Massee. The basidia were described as "cylindrical, bifurcate, aseptate, springing from the interlaced layer of hyphae at the apex of the stem, either contemporaneous with, or later than, the gonidiophores."

Such was the state of knowledge regarding this plant when

Professor Burt presented the paper mentioned in the first paragraph. His conclusions may be summarized as follows: "Specimens of the *Dacryopsis* collected in August, October, November and December show only the conidial condition, and no true basidia and basidiospores. Until the presence of basidia is demonstrated, *Graphium giganteum* (Pk.) Sacc. should be regarded as a conidial rather than a basidiomycetous stage of the ascomycete *Lecanidion leptospermum* (Pk.) Sacc." My own results only confirm those of Professor Burt. I have examined many specimens of the *Graphium* from several localities, and of various ages, and have made careful paraffin sections, but as yet I have found no structure which might be interpreted as a basidium.

In the autumn of 1900, specimens of the *Holwaya* were found growing on a rotten basswood log in woods not far from Ithaca. A few days later others were found on oak in the same woods. The *Graphium* was present in both cases. Inasmuch as it seemed desirable to determine definitely, if possible, the relation between the two forms, cultures were made from the specimens on oak in the following manner: Agar was prepared using a decoction of dead oak bark as a nutrient base. A bit of the hymenium was then taken up with a sterilized scalpel, and crushed in boiled water on a flamed slide. Dilution cultures were made on acidified medium in the usual manner. The same was done with the conidia. Ascospores were present in considerable numbers, and could easily be recognized in the agar, with the aid of the microscope. The cultures were examined at intervals for two or three days, but no signs of germination appearing, they were, unfortunately, neglected for a day or two. When next they were examined, numerous small colonies were present in the cultures of the ascospores. Examination with the microscope showed that the spore could still be made out in the center of many of the colonies, with threads actually attached to it, although some of the colonies were so far advanced that the spore was obliterated. There could be no doubt, therefore, that the colonies came from the ascospores. The cultures were allowed to stand a week, when it was found that plate number two of the set contained a pure culture, except for a single colony of *Penicillium*. Some of the colonies were then carefully transferred to

sterilized bean stems, and dead oak stems and bark. On the bean stems growth was very slow, but after a week, mycelial threads could be seen extending into the liquid at the bottom of the test-tube. Soon these formed a felty mass on the surface of the water. After two weeks minute dark points appeared on the bean stems. These rapidly increased in size, until they assumed all the appearances of normal *Graphium giganteum*, except that they were smaller than those grown under natural conditions. This was to be expected. Examination with the microscope revealed conidia in great numbers, and of the normal form, borne on conidiophores likewise normal. No growth occurred on the oak bark. The original cultures of the conidia failed to produce germination. Attempts to repeat the germination of the ascospores also failed. As yet after more than three months no ascomata have appeared.

These cultures seem to remove all doubt that *Graphium giganteum* (Pk.) Sacc., and the ascomycete forming the genus *Holwaya* are different stages in the development of the same plant. The question now arises, What shall this plant be called? The first name applied to the *ascus* stage was *leptosperma* Peck. If that name be applied our plant would be called *Holwaya leptosperma* (Pk.) Durand. But if the first name applied to the plant *in any of its forms* be used, it should be called *Holwaya gigantea* (Pk.) Durand. The latter combination seems the more logical, and has the sanction of precedent. But what is the systematic position of the plant? It has already been placed in three different families, viz.: Pezizaceae, Patellariaceae and Bulgariaceae. Saccardo placed it in the last-named group on account of its "gelatinous stratum" mentioned in the first description of *Bulgaria ophiobolus* Ellis. I have examined a large number of plants, both fresh and dry, but I cannot detect any indication of a gelatinous nature; certainly none such as exists in *Bulgaria* or *Coryne*. On the contrary the substance is rather a fleshy-mealy when fresh, becoming hard and brittle when dry. The flesh is dark brown in color. A section shows that the paraphyses are longer than the asci, and cohere at the tips into a thick black epithecium covering the hymenium. These characters locate it in the Patellariaceae where Dr. Peck first placed it. This, I think, is its proper position. *Holwaya* is

nearest related to *Lahmia* Körb., from which it differs in its stipitate habit, dark hypothecium, and tomentose stem.

I add a description and synonymy.

HOLWAYA Saccardo, Syll. Fung. 8: 646. 1889

A genus of the Patellariaceae. Ascomata stipitate, stipe tomentose; hypothecium and excipulum dark brown; sporidia 8, hyaline, filiform, multiseptate, not breaking up at maturity.

Holwaya gigantea (Peck) Durand

Ascus form.

Patellaria leptosperma Peck, Reg. Rep. 30: 62. 1878.

Bulgaria ophiobolus Ellis Am. Nat. 17: 193. 1883.

Holwaya ophiobolus (Ell.) Sacc. Syll. Fung. 8: 646. 1889.

Lecanidion leptospermum (Pk.) Sacc. Syll. Fung. 8: 800. 1889.

Chlorosplenium Canadense E. & E. Proc. Phil. Acad. Nat. Sci. 41: 146. 1893.

Holwaya tiliacea E. & E. Am. Nat. 31: 427. 1897.

Conidial form.

Stilbum giganteum Peck, Reg. Rep. 24: 93. *pl. 3. f. 7-9.* 1871.

Coryne Ellisii Berk. Grev. 2: 33. 1873.

Graphium giganteum (Pk.) Sacc. Syll. Fung. 4: 611. 1886.

Dacryopsis Ellisiana Massee, Jour. Myc. 6: 181. *pl. 7. f. 19-21.* 1891.

Ascus form.—Caespitose or single, scattered, stipitate. Disk cup-shaped, becoming plane, or the margin reflexed and umbilicate, orbicular, or irregular from mutual pressure, when fresh .75–1.5 cm. in diameter, greenish-black, externally same color, pruinose or granular. Stem .25–.75 cm. high, tapering downward, greenish-black, covered with an olive-brown tomentum, which often disappears with age. Flesh dark brown to black. In drying the plant shrinks to less than one-half its former size and becomes black. Hypothecium well-developed, of intricately interwoven hyphae; excipulum of slender interwoven hyphae, passing into a cortical layer of pseudo-parenchyma, of polygonal cells about 10 μ in diameter, which project from the surface in groups giving it a granular appearance. This layer is confined to the sides of the cup and upper part of the stem. Stem composed of closely interwoven hyphae which project from the surface forming the tomen-

tum of septate, sparingly branched threads, $2\ \mu$ thick. Asci narrowly and evenly clavate, apex rounded, not blue with iodine, variable in size in the same individual, $120-200 \times 10-12\ \mu$. Sporidia 8, fascicled or multiseriate in the ascus, filiform-cylindrical, or very narrowly clavate-cylindrical, ends rounded, or sometimes acute at one end, straight, curved, or slightly sigmoid, hyaline, multinucleate, becoming 14-20-septate into cells about as long as wide, very variable in size, $30-75 \times 3-4\ \mu$. Paraphyses filiform, slender, longer than the asci, globose at the tips which cohere, and with amorphous matter form the epithecium.

Conidial form.—Gregarious or single, fleshy-gelatinous, stem cylindrical or tapering upward, $3-10 \times 2\ \text{mm.}$, black. Head broadly elliptical, soft, viscid, pallid, $2-6 \times 2-4\ \text{mm.}$ Conidiophores very slender, branched. Conidia hyaline, elliptical, $3 \times 1\ \mu$.

On prostrate rotten logs, usually in crevices and depressions in the bark, also on the bare wood, oftenest on *Tilia*, but also on *Acer*, *Quercus* and *Magnolia*. October and November.

Vermont (Burt)!; New York (Peck!, Clinton, Durand!); Ohio (Kellerman)!; W. Virginia (Nuttall!); Iowa (Holway!); Canada (Macoun, Dearness!).

Explanation of Plate

Figures 2, 4, 5, 6, 7 and 8 were drawn to the same scale. Figures 1-4 are from specimens collected at Ithaca, October, 1900.

1. Longitudinal section of whole ascoma to show form and tomentose stem.
2. A portion of the tomentum, showing connection of the threads with the hyphae of the stem.
3. A longitudinal section of the cup a short distance from the margin.
4. Asci, paraphysis and sporidia.
5. Ascus and three sporidia of *Chlorosplenium Canadense* E. & E., from specimens collected at London, Canada, by Professor Dearness (no. 2032 B).
6. Asci, paraphysis and sporidia of *Lecanidion leptospermum* (Pk.) Sacc., from specimens collected by Dr. Peck, at Copake, N. Y.
7. Ascus, paraphysis and sporidia of *Holwaya tiliacea* E. & E., from specimens collected at Middlebury, Vt., by Professor Burt.
8. Ascus, paraphysis and sporidia of *Holwaya ophiobolus* (Ell.) Sacc., from specimens in Ellis's N. A. F., no. 996.

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